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Peripheral Mechanisms Contributing to the Glucocorticoid Hypersensitivity in *Pomc* null mice treated with Corticosterone.

Running Title: Glucocorticoid action in *Pomc*-null mice

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Abbreviations: Agt, angiotensinogen**;** CORT, corticosterone-supplemented water; GC, glucocorticoid; NEFA, non esterified fatty acids; GR, glucocorticoid receptor; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; POMC, proopiomelanocortin; RAS, renin-angiotensin system.

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ABSTRACT

Pro-opiomelanocortin (POMC)-deficiency causes severe obesity through hyperphagia of hypothalamic origin. However, low glucocorticoid levels caused by adrenal insufficiency mitigates against insulin resistance, hyperphagia and fat accretion in *Pomc*$^{-/-}$ mice. Upon exogenous glucocorticoid replacement, (CORT) *Pomc*$^{-/-}$ mice show exaggerated responses including excessive fat accumulation, hyperleptinaemia and insulin resistance. To investigate the peripheral mechanisms underlying this glucocorticoid hypersensitivity we examined the expression levels of key determinants and targets of glucocorticoid action in adipose tissue and liver. Despite lower basal expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which generates active glucocorticoids within cells, CORT-mediated induction of 11β-HSD1 mRNA levels was more pronounced in adipose tissues of *Pomc*$^{-/-}$ mice. Similarly, CORT treatment increased lipoprotein lipase mRNA levels in all fat depots in *Pomc*$^{-/-}$ mice, consistent with exaggerated fat accumulation. Glucocorticoid receptor (GR) mRNA levels were selectively elevated in liver and retroperitoneal fat of *Pomc*$^{-/-}$ mice but were corrected by CORT in the latter depot. In liver, CORT increased PEPCK mRNA levels specifically in *Pomc*$^{-/-}$ mice, consistent with their insulin resistant phenotype. Furthermore, CORT induced hypertension in *Pomc*$^{-/-}$ mice, independently of adipose or liver renin-angiotensin system (RAS) activation. These data suggest CORT-inducible 11β-HSD1 expression in fat contributes to the adverse cardiometabolic effects of CORT in POMC deficiency, whereas higher GR levels may be more important in liver.

INTRODUCTION

Glucocorticoids exert pleiotropic effects on metabolism and energy partitioning. Centrally, they increase food intake and reduce energy expenditure whilst peripherally they promote insulin resistance, fat accumulation (Dallman *et al*. 1993; Kellendonk *et al*. 2002) and hypertension (Saruta 1996; Whitworth *et al*. 2001). Polymorphisms in the human glucocorticoid receptor NR3c1 gene (GR) are associated with glucocorticoid hypersensitivity, visceral obesity, hypertension and increased cardiovascular disease risk (Buemann *et al*. 1997; Rosmond *et al*. 2000; Ukkola *et al*. 2001; Dodson *et al*. 2001; van Rossum *et al*. 2003). Many rodent models of obesity are characterised by hypercorticosteronemia, with weight gain normalised following adrenalectomy and reinstated by glucocorticoid replacement (Debons *et al*.1982; Freedman *et al*. 1986; Sainsbury *et al*.1997; Makimura *et al*. 2000). Although plasma glucocorticoid levels are normal in human idiopathic obesity (Flier 2004), it has been proposed that intra-adipose glucocorticoid action is selectively increased, through increased adipose expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), the intracellular enzyme that regenerates active glucocorticoids from intrinsically inert 11keto-glucocorticoids (Andrew *et al*. 2002; Jamieson *et al*. 2000; Kotelevtsev *et al*. 1997).

Obese humans (Rask *et al*. 2001; Paulmyer-Lacroix *et al*. 2002; Lindsay *et al*. 2003; Kannisto *et al*. 2004) and some rodent models of obesity (Masuzaki *et al*. 2001; Livingstone *et al*. 2000) have selectively increased adipose levels of 11β-HSD1 and transgenic over-expression of 11β-HSD1 in adipocytes causes hyperphagia, obesity, insulin resistance and hypertension despite unchanged systemic glucocorticoid levels (Kotelevtsev *et al*. 1997; Masuzaki *et al*. 2003). Hepatic over-expression of 11β-

HSD1 has no effect on adiposity, but causes hypertension and insulin resistance (Paterson *et al*. 2004). Conversely, mice deficient in 11β-HSD1 are insulin sensitised and resist the adverse metabolic effects of a high fat diet (Kotelevtsev *et al*. 1997; Morton *et al*. 2001; Morton *et al*. 2004).

Proopiomelanocortin (POMC) is a polypeptide precursor which undergoes extensive postranslational modification to yield a range of smaller, biological active peptides. These include α-, β- and γ melanocyte stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH), collectively known as the melanocortins. Inactivating mutations of the POMC gene in humans and mice results in a complex phenotype. Loss of melanocortin signalling within the hypothalamus cause hyperphagia and obesity (Krude *et al*. 1998; Yaswen *et al*.1999; Challis *et al*. 2004). Further, a failure to produce ACTH within the anterior pituitary causes adrenal insufficiency with low or absent circulating glucocorticoids (Krude *et al*. 1998; Yaswen *et al*.1999; Challis *et al*. 2004). *Pomc*$^{-/-}$ mice are therefore unusual amongst rodent models in that obesity develops in the absence of circulating glucocorticoids. However, glucocorticoid treatment exacerbates hyperphagia and obesity in adult *Pomc*$^{-/-}$ mice and induces severe insulin resistance, hyperleptinemia and diabetes (Coll *et al*. 2005).

We have tested the hypothesis that increased glucocorticoid action in peripheral tissues of glucocorticoid-treated *Pomc*$^{-/-}$ mice contributes to their apparent glucocorticoid-hypersensitivity and exaggerated metabolic syndrome-like phenotype. We further demonstrate that glucocorticoid replacement induces hypertension in *Pomc*$^{-/-}$ mice, independently of RAS activation.

MATERIALS AND METHODS

Animals and corticosterone (CORT) replacement. The generation of *Pomc*$^{-/-}$ mice on a 129/SvEv background has been described previously (Challis *et al*. 2004). All mice were housed in standard conditions on a 12h light: 12h dark cycle (lights on 7am) with *ad libitum* access to water and chow (4.5% fat diet, Special Diet Services, Witham, UK). Eight week-old male mice (n=5/group) were treated with corticosterone (25μg/ml) in their drinking water, a dose that results in similar plasma glucocorticoid levels and hypothalamic CRH mRNA levels in *Pomc*$^{-/-}$ and wild type mice (Coll *et al*. 2005). All animal protocols used in these studies were approved under the auspices of the UK Home Office Animals (Scientific Procedures) Act, 1986.

Blood Pressure Measurement. Systolic blood pressure was measured photoelectrically in the tail of restrained conscious mice using an IITC model 179 analyser (Woodland Hills, California, USA). Prior to recording measurements, all mice underwent 3 periods of training to accustom them to the procedure. Mice were warmed at 32^{o}C for 30 min before taking 10 consecutive readings. The first five were discounted and a mean value of systolic blood pressure was calculated from the last five readings. Five mice from each treatment group were measured. All analog recordings were analysed by an independent observer who was blinded to the genotype of the mice and any treatment they had received.

Plasma hormone and lipid measurements. Animals were killed between 0800 and 0900h by cervical dislocation. Trunk blood samples were collected into EDTA coated tubes (Sarstedt, Germany), centrifuged (6000g x 10min) and plasma stored at -80^{o}C

until required for assay. Non-esterified fatty acid (NEFA) and triglyceride levels were determined by commercial kits (NEFA, Roche Diagnostics, West Suffolk, UK; triglyceride, Dade Behring, Marburg, Germany). Plasma renin and angiotensinogen concentrations were determined as previously described (Morton *et al*. 2005).

Tissue Morphology and hepatic triglyceride levels. Neutral lipids, cholesterol and fatty acids were identified by light microscopy at x 40 magnification in cryostat liver sections (30μm) stained with Oil red O (Sigma) and counter-stained with haematoxylin as previously described (Morton *et al*. 2005). Hepatic triglycerides were extracted by homogenisation in isopropanol (10 volumes) then incubation for 45 min at 37^{o}C and measured spectrophotometrically in supernatants (3000g x 10 min) using reagent TR224221 (Alpha Laboratories).

RNA extraction and northern blot analysis. Pieces of liver and adipose tissues (inguinal, retroperitoneal and epididymal) were rapidly frozen in dry ice, stored at -80^{o}C then homogenised in Trizol (Invitrogen, UK). Total RNA was purified using a binding matrix (RNaid Plus kit, BIO 101; Anachem, UK) and eluted in diethylpyrocarbonate-treated water containing 400 U/ml RNasin (Promega, Southampton, UK) and 10mmol/l dithiothreitol. RNA (5-10μg) was blotted and hybridized to ^{32}P-labelled cDNA probes for mouse 11β-HSD1, GR, angiotensinogen, phosphoenolpyruvate carboxykinase (PEPCK), lipoprotein lipase (LPL) and 18S as previously described (Morton *et al*. 2005). Specific mRNAs were quantified using a phosphorimager (Fuji BAS FLA 2000) and Aida image analysis software (Raytek, Sheffield, UK) and are expressed in arbitrary units (A.U.) relative to 18S RNA.

Statistical Analyses. The effects of genotype and corticosterone interactions were assessed by 2-way ANOVA followed by post-hoc Tukeys' tests for group differences. Significance was set at $p<0.05$. Values are means ±SEM.

RESULTS

Pomc$^{-/-}$ mice have reduced intra-adipose GC action but exaggerated CORT mediated GC amplification.

Corticosterone-treated *Pomc*$^{-/-}$ and wild type mice had similar plasma corticosterone levels and hypothalamic CRH mRNA levels (Coll *et al*. 2005). To examine potential mechanisms of CORT hypersensitivity in *Pomc*$^{-/-}$ mice, 11β-HSD1 and GR mRNA levels were measured in epididymal, inguinal and retroperitoneal adipose depots. Adipose 11β-HSD1 mRNA expression was lower in all untreated *Pomc*$^{-/-}$ compared to wild type mice (Fig. 1A) and was dramatically increased by CORT in both genotypes (Fig. 1A), with larger increases (2 to 4-fold greater) in *Pomc*$^{-/-}$ mice.

Adipose expression of GR mRNA was higher in the retroperitoneal fat of *Pomc*$^{-/-}$ mice and restored to wild type levels by CORT treatment (Fig. 1B). GR mRNA levels did not differ in inguinal and epididymal fat between *Pomc*$^{-/-}$ and wild type mice, and were unaffected by CORT treatment in either genotype (Fig. 1B).

To investigate mechanisms downstream of 11β-HSD1/GR by which CORT-treatment selectively increases fat mass in *Pomc*$^{-/-}$ mice, adipose levels of mRNA encoding lipoprotein lipase (LPL), a glucocorticoid-regulated gene (Fried *et al*.1993), were measured. Although LPL mRNA levels were the same in untreated *Pomc*$^{-/-}$ and wild type mice in all depots, adipose LPL expression in *Pomc*$^{-/-}$ mice was markedly increased by CORT-treatment (Fig. 1C) consistent with increased triglyceride uptake, and fat mass in *Pomc*$^{-/-}$ mice. In wild type mice, CORT-treatment increased LPL mRNA only in the inguinal depot, and to a lesser extent than in *Pomc*$^{-/-}$ mice (Fig.

1C), suggesting adipose depot-dependent regulation of LPL by glucocorticoids in non-obese mice, consistent with previous data in rats (Freedman *et al*. 1986).

Phosphoenolpyruvate carboxykinase (PEPCK) is an enzyme essential for gluconeogenesis in liver and for glycerol synthesis in adipose tissue (Pilkis & Granner 1992; Reshef *et al*. 2003). PEPCK is a classical glucocorticoid target gene which is positively regulated by glucocorticoids in hepatocytes and negatively regulated in adipocytes (Sasaki *et al*.1984; Nechushtan *et al*. 1987). Consistent with this, adipose PEPCK mRNA levels were decreased in epididymal and retroperitoneal fat by CORT-treatment in wild type mice (Fig. 1D). Surprisingly, given their glucocorticoid deficiency, *Pomc*$^{-/-}$ mice had lower levels of PEPCK mRNA in adipose tissue than wild type (Fig. 1D). However, although CORT treatment in *Pomc*$^{-/-}$ mice decreased PEPCK expression in inguinal and retroperitoneal adipose tissue (significantly lower than in CORT-treated wild type mice; p=0.01), it had no effect on PEPCK mRNA levels in epididymal adipose tissue, suggesting that other regulatory factors dominate PEPCK expression in adipose tissue of *Pomc*$^{-/-}$ mice (Fig. 1D).

Pomc$^{-/-}$ mice are dyslipidaemic, and have unaltered hepatic 11β-HSD1 but higher GR mRNA levels.

Hepatic 11β-HSD1 mRNA levels were similar between the two genotypes (Fig. 2A) and unaffected by CORT (Fig. 2A). Hepatic GR mRNA levels were higher in *Pomc*$^{-/-}$ compared to wild type mice (Fig. 2B), but again CORT had no effect on GR mRNA levels (Fig. 2B).

Hepatic PEPCK expression was lower in *Pomc*$^{-/-}$ than in wild type mice (Fig. 2C) and was increased by CORT treatment to levels equivalent to untreated wild type mice. In contrast, CORT decreased hepatic PEPCK mRNA levels in wild type mice (Fig.2C).

Pomc$^{-/-}$ mice showed markedly higher circulating triglyceride levels (Fig. 3A) and hepatic lipid accumulation than wild type mice (Fig. 3B), with 6-fold higher levels of hepatic triglyceride (P<0.001) (Fig. 3C). However, CORT had no effect on plasma triglyceride levels in either genotype (Fig. 3A), nor did it worsen the liver phenotype (Fig. 3C). *Pomc*$^{-/-}$ and wild type mice had similar plasma NEFA levels which were unaffected by CORT (Fig. 3D).

CORT drives hypertension in *Pomc*$^{-/-}$ mice independently of adipose and liver RAS activation.

Pomc$^{-/-}$ mice had similar blood pressure to wild type mice (Fig. 4A). CORT markedly increased blood pressure only in *Pomc*$^{-/-}$ mice (Fig. 4A). Since hypertension following transgenic expression of 11β-HSD1 in adipose or liver is associated with increased levels of angiotensinogen in each of these tissues, respectively (Masuzaki *et al*. 2001; Paterson *et al*. 2004) we hypothesised that a similar mechanism may drive CORT-mediated hypertension in *Pomc*$^{-/-}$ mice. We therefore examined key components of the renin-angiotensin system (Guyton 1991). *Pomc*$^{-/-}$ mice had higher hepatic angiotensinogen mRNA levels than controls (Fig. 4B). However, CORT did not alter hepatic angiotensinogen mRNA levels in either genotype (Fig. 4B). Consistent with lower intra-adipose GC action, adipose angiotensinogen mRNA levels were lower in *Pomc*$^{-/-}$ mice in all adipose depots (Fig. 4C). CORT increased angiotensinogen mRNA levels specifically in epididymal adipose tissue of both genotypes (2 fold increase;

$P<0.001$) (Fig. 4C) but had no effect on angiotensinogen mRNA levels in inguinal or retroperitoneal adipose tissue of either genotype (Fig. 4C). Plasma angiotensinogen concentrations did not differ with genotype or CORT (Fig. 4D). As has been found in another model of glucocorticoid deficient obesity (Morton *et al*. 2005), plasma renin concentration was markedly higher in *Pomc*$^{-/-}$ mice (Fig. 4E) but this was unaffected by CORT (Fig. 4E).

DISCUSSION

Increased glucocorticoid action specifically in adipose (Masuzaki *et al*. 2001) or liver (Paterson *et al*. 2004) produce distinct metabolic syndromes with hypertension. Increased glucocorticoid receptor sensitivity is also associated with altered fat distribution, hypertension and cardiometabolic disease (Buemann *et al*. 1997; Rosmond *et al*. 2000; Ukkola *et al*. 2001; Dodson *et al*. 2001; van Rossum *et al*. 2003). We hypothesised that altered tissue regeneration of active glucocorticoid and/or peripheral tissue sensitivity to GCs might explain in part the exaggerated fat accumulation, insulin resistance (Coll *et al*. 2005) and the hypertension observed in *Pomc*$^{-/-}$ mice with glucocorticoid replacement.

With fixed circulating glucocorticoid levels, 11β-HSD1 and GR expression levels are the key determinants of GC action. *Pomc*$^{-/-}$ mice had lower adipose but similar hepatic levels of 11β-HSD1 mRNA levels to wild type mice. CORT-treatment dramatically and more markedly increased 11β-HSD1 in the adipose tissue of *Pomc*$^{-/-}$ mice. This was accompanied by a marked increase in the expression of the glucocorticoid inducible (Fried *et al*. 1993) gene LPL, which is consistent with the exaggerated accumulation of fat in these mice. Intriguingly, these data suggest that, at least in adipose tissue, 11β-HSD1 itself is a glucocorticoid target gene. This finding is consistent with most (Jamieson *et al*. 1995; Voice *et al*. 1996; Hammami & Siteri 1991; Bujalska *et al*. 1999), but not all (Napolitano *et al*. 1998) previous reports of glucocorticoid induction of 11β-HSD1 in a variety of cell types. Although not specifically measured here, increased adipose 11β-HSD1 activity is predicted to selectively amplify intra-adipose glucocorticoid concentrations, particularly when circulating levels of substrate are high. On the other hand, our data suggest that

congenital glucocorticoid deficiency has little impact upon hepatic 11β-HSD1 levels *in vivo* and is not regulated by corticosterone. In contrast, 11β-HSD1 mRNA levels are highly and positively regulated by glucocorticoids in adipose tissue.

GR levels are another major determinant of cellular glucocorticoid sensitivity (Vanderbilt *et al*. 1987; Geley *et al*. 1996). Small differences in GR mRNA levels can markedly alter glucocorticoid responsiveness (Geley *et al*. 1996; Reichardt *et al*. 2000). *Pomc*$^{-/-}$ mice had elevated GR mRNA levels in liver and retroperitoneal adipose tissue, suggesting increased glucocorticoid sensitivity selectively in these depots. Following CORT replacement in *Pomc*$^{-/-}$ mice, GR mRNA levels were restored to wild type levels in retroperitoneal adipose tissue but not in liver, consistent with tissue- and time-specific differences in GR autoregulation (Reichardt *et al*. 2000; Sheppard *et al*. 1990; Holmes *et al*. 1990; Holmes *et al*. 1995; Holmes *et al*. 1997; Kalinyak *et al*. 1987; Dong *et al*. 1988).

CORT had no additional effects on the hypertriglyceridaemia and fatty liver of the *Pomc*$^{-/-}$ mice, and did not affect plasma NEFAs, which were normal in *Pomc*$^{-/-}$ mice. The CORT-driven caloric excess in *Pomc*$^{-/-}$ mice may drive a further increase in the flux of triglycerides from the liver, that, coupled with increased adipose uptake via LPL, maintains the circulating and liver triglyceride levels constant and is consistent with increased adipose tissue mass in CORT treated *Pomc*$^{-/-}$ mice (Coll *et al*. 2005).

Adipose PEPCK is critical for glyceroneogenesis and is thus a key regulator of the level of fatty acid re-esterification (reviewed in (Reshef *et al*. 2003)). Unexpectedly, since glucocorticoids reduce adipose PEPCK, glucocorticoid deficient *Pomc*$^{-/-}$ mice

had lower levels of PEPCK mRNA in all adipose depots. This was further decreased by CORT treatment. The lower level of PEPCK mRNA in untreated *Pomc*$^{-/-}$ mice may be due to their higher fed blood glucose levels (Nechushtan *et al*. 1987; Opherk *et al*. 2004), thus reducing the need for glyceroneogenesis to generate glycerol-phosphate for fatty acid re-esterification. *Pomc*$^{-/-}$ mice have lower hepatic expression of PEPCK. This may not be due to lack of glucocorticoid signalling in liver, as mice with a liver-specific knock-out of GR have normal levels of PEPCK in liver (Opherk *et al*. 2004), but may be related to the higher circulating levels of insulin in *Pomc*$^{-/-}$ mice compared to wild type (Coll *et al*. 2005). Insulin dominantly and negatively suppresses hepatic PEPCK in the fed state (Pilkis & Granner 1992). In CORT-treated wild type mice, the repressive effect of insulin predominated. Indeed, the decreased levels of PEPCK in these mice compared to untreated wild type mice may reflect the increase in insulin levels following CORT (Coll *et al*. 2005). In contrast, hepatic PEPCK mRNA levels doubled following CORT treatment in *Pomc*$^{-/-}$ mice, consistent with hepatic insulin resistance and marked hyperinsulinaemia (Coll *et al*. 2005).

Blood pressure in *Pomc*$^{-/-}$ mice is normal despite their hypoadrenal state. This implies that secondary mechanisms are invoked to maintain cardiovascular function when circulating aldosterone and corticosterone concentrations are chronically reduced (Coll *et al*. 2004). It seems likely that the increased renin activity which we have observed in *Pomc*$^{-/-}$ mice is part of this adaptive process. However, corticosterone replacement did not normalise renin activity and selectively increased blood pressure in *Pomc*$^{-/-}$ mice. This was not attributed to a further activation of the circulating renin-angiotensin system, since neither renin nor its substrate angiotensinogen were increased. Indeed expression of angiotensinogen mRNA in liver and adipose tissues

did not correlate with blood pressure. It seems likely that corticosterone augmented existing mechanisms that were already sustaining vascular function. Apart from renin, these secondary processes are likely to involve the hyperinsulinaemic (Sowers 2004) state of *Pomc*$^{-/-}$ mice (which is exacerbated by corticosterone treatment) (Coll *et al*. 2005), the sympathetic nervous system (Rascher *et al*. 1979) (which is thought to explain glucocorticoid-induced hypertension in normal mice) or structural adaptation of the vasculature (Wallerath *et al*. 2004).

In summary, we show that increased adipose tissue-specific sensitivity to glucocorticoids in *Pomc*$^{-/-}$ mice may result in part from exaggerated induction of 11β-HSD1 in adipose tissue with CORT administration. Whilst acknowledging that mRNA changes do not always translate to altered protein (or enzyme activity) levels, these data nevertheless suggest that 11β-HSD1 might be a more potent mediator of intra-adipose GC action than the glucocorticoid receptor levels whereas in liver, higher GR levels contribute to the diabetogenic phenotype of the *Pomc*$^{-/-}$ mice.

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Figure Legends

FIG. 1. ***Mediators of GC action in adipose tissue of Pomc$^{-/-}$ mice and effects of CORT-treatment on GC target genes***

(A) Representative northern blot showing levels of 11β-HSD1 mRNA and 18S RNA in epididymal adipose tissue of *Pomc*$^{-/-}$ (-/-) and wild type (+/+) mice, either untreated or treated for 10 days with corticosterone (cort). (B-E) Quantitation of adipose tissue-specific 11β-HSD1 (B), GR (C), LPL (D) and PEPCK (E) mRNA levels in experimental mice. Epi, epididymal fat; ing, inguinal fat; retro, retroperitoneal fat. Data are presented as % of the value in untreated wild type mice (100%) and are the means ± SEM; n=5/group. Significance, **P*<0.05, ***P*<0.01 and ****P*<0.001.

FIG. 2. ***Mediators of GC action in liver of Pomc$^{-/-}$ mice and effects of CORT-treatment on GC target genes***

Liver mRNA expression of (A) 11β-HSD1 (B) GR and (C) PEPCK in *Pomc*$^{-/-}$ (-/-) and wild type (+/+) mice, either untreated or treated for 10 days with corticosterone (cort). Data are presented as % of the value in untreated control mice (100%) and are the means ±SEM; n=5/group. Significance, **P* <0.05, ***P*<0.01 and ****P*<0.001.

FIG. 3. ***Dyslipideamia and fatty liver in Pomc$^{-/-}$ mice.***

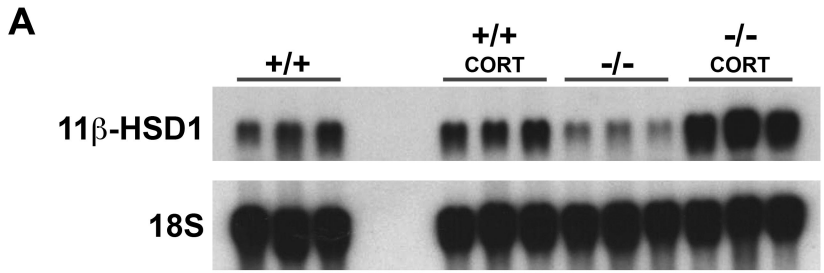
(A) Plasma triglyceride levels in *Pomc*$^{-/-}$ (-/-) and wild type (+/+) mice, either untreated or treated for 10 days with corticosterone (cort). (B) Oil Red O staining of neutral lipid in liver sections of wild type mice (+/+, left upper panel), CORT-treated wild type mice (+/+, left bottom panel), *Pomc*$^{-/-}$ (-/-, right upper panel) and CORT-treated *Pomc*$^{-/-}$ (-/-, right bottom panel). Magnification is x40; red=Oil red O, blue = haematoxylin (nuclei). (C) hepatic triglyceride content in *Pomc*$^{-/-}$ (-/-) and wild type

(+/+) mice, either untreated or treated for 10 days with corticosterone (cort). (D) plasma levels of non esterified fatty acids (NEFA) in *Pomc*$^{-/-}$ (-/-) and wild type (+/+) mice, either untreated or treated for 10 days with corticosterone (cort). Data are means ± SEM; (n=6/group). Significance, ***P<0.001.

FIG 4. ***Corticosterone treatment increases blood pressure in Pomc$^{-/-}$ mice: effect of CORT-treatment on the Renin-angiotensin system***

Effect of 10 days corticosterone treatment (cort) on (A) systolic blood pressure (B) renin concentration (C) plasma angiotensinogen (D) angiotensinogen (Agt) mRNA in adipose tissue (AT) and (E) angiotensinogen (Agt) mRNA levels in liver in wild type (+/+) and *Pomc*$^{-/-}$ (-/-) mice. Epi, epididymal fat; ing, inguinal fat; retro, retroperitoneal fat. Data are the means ± SEM, and for transcript levels are expressed relative to levels in untreated wild type mice (100%); n=5/group. Significance; *P<0.05, **P<0.01 and ***P<0.001.

Figure 1


A
+/+
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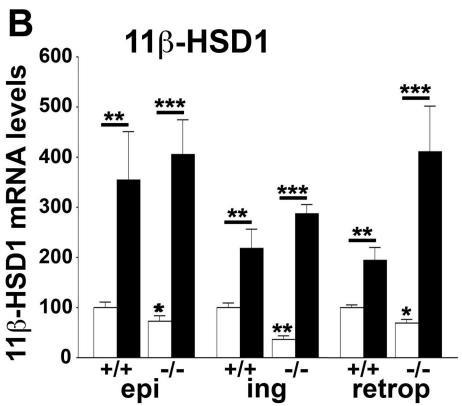
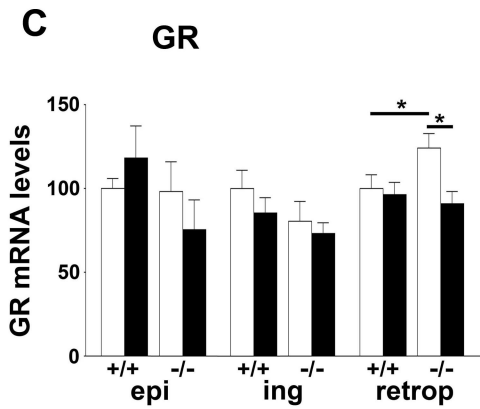
-/-
-/- CORT
11β-HSD1
18S


B
11β-HSD1
11β-HSD1 mRNA levels
600
500
400
300
200
100
0
+/+ -/- +/+ -/- +/+ -/-
epi
ing
retrop


C
GR
GR mRNA levels
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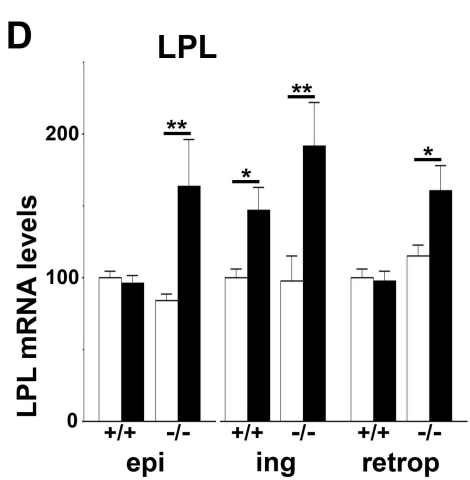
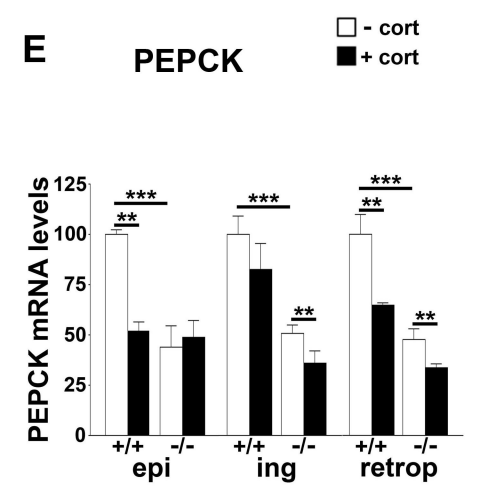
100
50
0
+/+ -/- +/+ -/- +/+ -/-
epi
ing
retrop


D
LPL
LPL mRNA levels
200
100
0
+/+ -/- +/+ -/- +/+ -/-
epi
ing
retrop


E
PEPCK
- cort
+ cort
PEPCK mRNA levels
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100
75
50
25
0
+/+ -/- +/+ -/- +/+ -/-
epi
ing
retrop

Figure 2

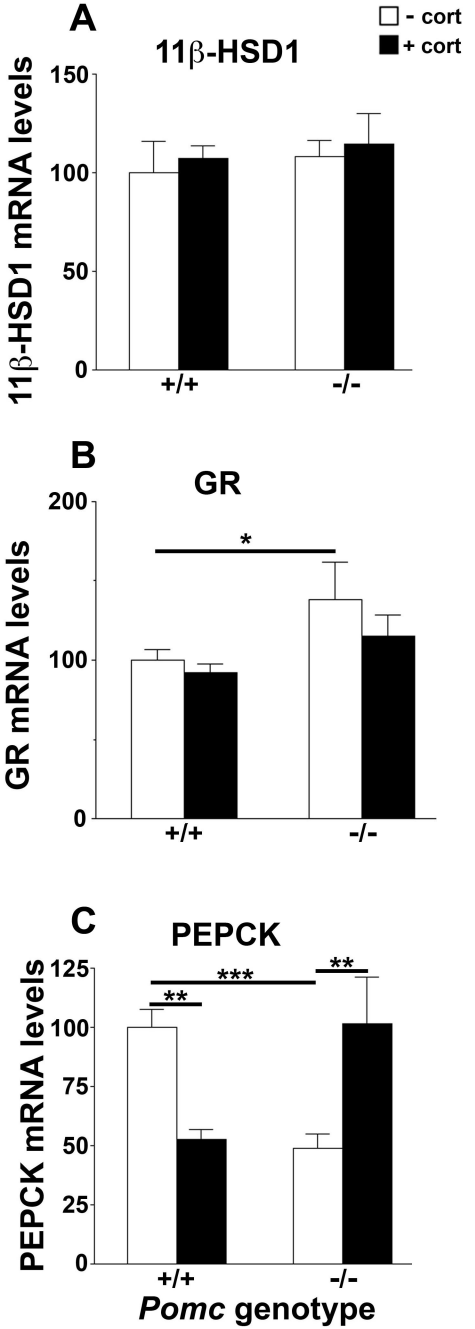

A
11β-HSD1
- cort
+ cort
11β-HSD1 mRNA levels
150
100
50
0
+/+
-/-
B
GR
*
GR mRNA levels
200
100
0
+/+
-/-
C
PEPCK
**
***
**
PEPCK mRNA levels
125
100
75
50
25
0
+/+
-/-
Pomc genotype

Figure 3

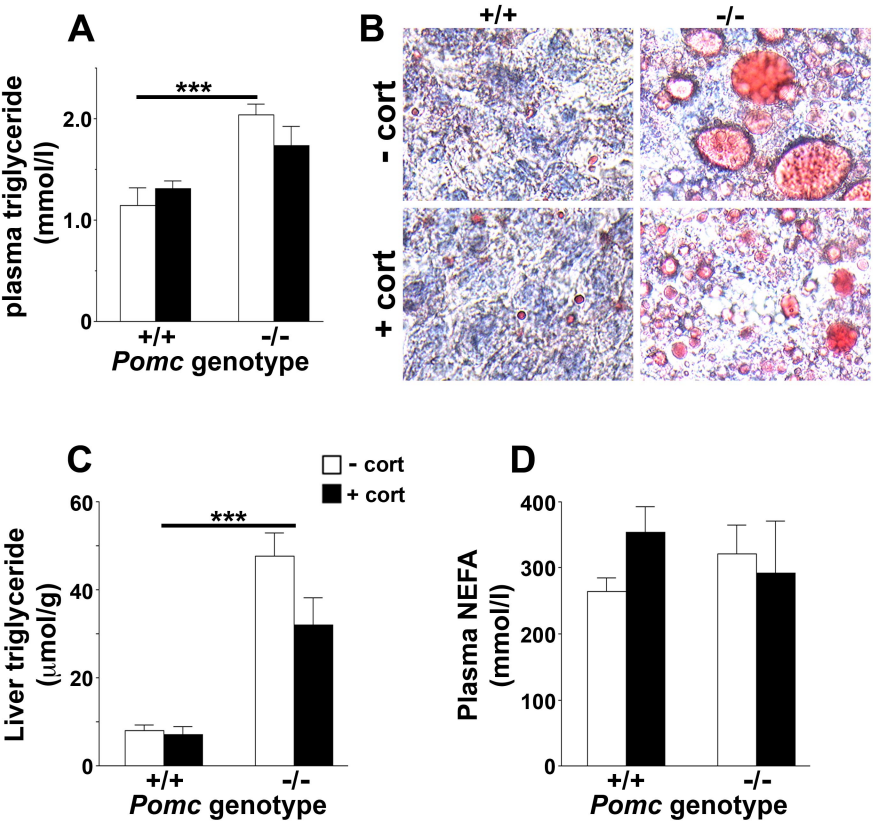
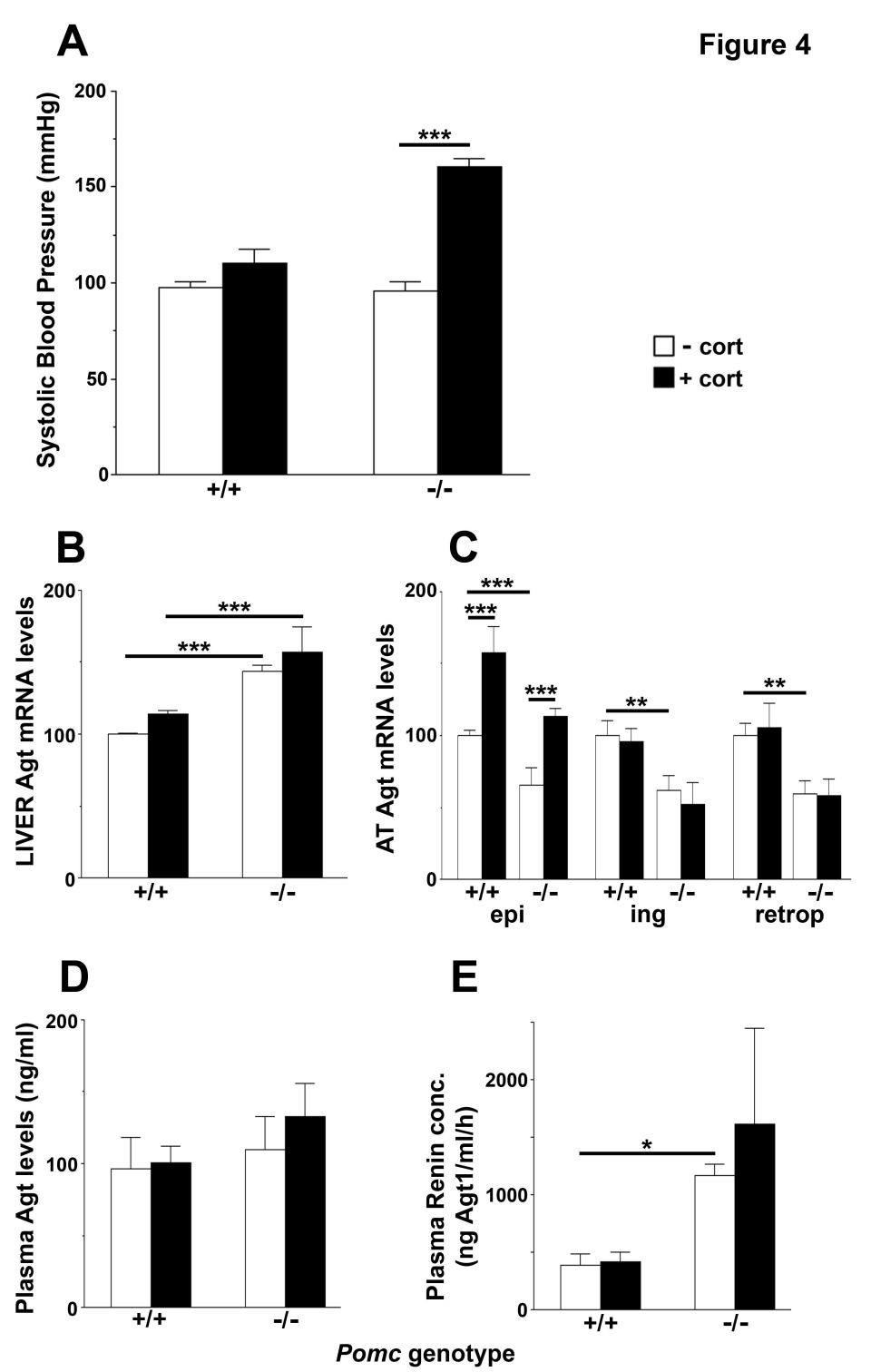

A
plasma triglyceride (mmol/l)
***
2.0
1.0
0
+/+
-/-
Pomc genotype
B
+/+
-/-
- cort
+ cort
C
- cort
+ cort
Liver triglyceride (μmol/g)
60
40
20
0
***
+/+
-/-
Pomc genotype
D
Plasma NEFA (mmol/l)
400
300
200
100
0
+/+
-/-
Pomc genotype

Figure 4


A
Systolic Blood Pressure (mmHg)
200
150
100
50
0
***
+/+
-/-
- cort
+ cort
B
LIVER Agt mRNA levels
200
100
0
***
***
+/+
-/-
C
AT Agt mRNA levels
200
100
0
***
***
***
**
**
+/+
-/-
+/+
-/-
+/+
-/-
epi
ing
retrop
D
Plasma Agt levels (ng/ml)
200
100
0
+/+
-/-
E
Plasma Renin conc. (ng Agt1/ml/h)
2000
1000
0
*
+/+
-/-
Pomc genotype